



# Methods for the analysis of submicrometer- and nanoplastic particles in the environment

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## ABSTRACT

Nanoplastic is an emerging topic of relevance in environmental science. The analytical methods for microplastic have a particle size limit of a few micrometers so that new methods have to be developed to cover the nanometer range. This contribution reviews the progress in environmental nanoplastic analysis and critically evaluates which techniques from nanomaterial analysis may potentially be adapted to close the methodological gap. A roadmap is brought forward for the whole analytical process from sample treatment to particle characterization. This includes a critical review of (i) methods for analyte extraction and preconcentration from various environmental matrices; (ii) methods for the separation of the nanoplastic into specific size fractions; (iii) light scattering techniques and various types of microscopy to characterize the particle fractions; (iv) chemical identification of particles to validate the obtained data. For these methods, we will discuss prospects and limitations to develop analytical protocols for specific sampling scenarios.

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## 1. Introduction

Environmental plastic and especially microplastic (MP) pollution is a topic of great public concern. In recent years, questions concerning even smaller particles, so-called nanoplastic, have emerged and are of pressing interest, especially since it has been identified in facial scrubs [1] and in marine surface waters [2]. MP is defined as particles in the size range from 1  $\mu\text{m}$  to 5 mm, nanoplastic ranges from 1 nm to 100 nm and the sizes between 100 nm and 1  $\mu\text{m}$  are called sub $\mu$ -plastic [3]. This classification adheres to the European Commission's definitions for engineered nanoparticles (ENPs) [4]. However, there is still debate on the size classes for nanoplastic, with an alternative that defines the whole nanometer range (1 nm–1000 nm) as nanoplastic [5]. Furthermore, MP is separated into primary MP, which comprises particles that were produced for a specific purpose (e.g. for cosmetics), and secondary MP, which is generated from larger plastic debris by fragmentation [3]. This classification could be applied to sub $\mu$ - and nanoplastic as well, denoting e.g. a polystyrene (PS) latex or nanometer-sized plastic particles in cosmetics as primary nanoplastic. Particles

that originate from larger pieces by fragmentation in the environment would, then, be classified as secondary nanoplastic. This topic, thereby, creates a cross-section with nanoparticle science, because nanoplastic particles are in principle polymeric nanoparticles. It is, however, well placed in the field of environmental plastic analysis, since it is part of the whole plastic contamination problem. The debate on what actually is comprised by 'plastic', thereby meaning 'synthetic polymer' (including associated additives), is still ongoing. Usually, particle size and, implicitly, the prerequisite that the particle is insoluble in water are the core determinants for plastic particle analysis. However, this excludes important aspects like the chemical composition (polymer type, additives, ageing) or whether particles made of modified polymers of natural origin (e.g. natural rubbers in tire wear) can be assigned to 'plastic'. For more details on a definition and categorization framework for plastic debris, the reader is referred to a recent publication of Hartmann et al. [6].

These distinctions have to be kept in mind, when defining the analytical question for specific sample scenarios. In MP analysis, the sampling and sample treatment of MP is accomplished with respect to the system that is probed. Nets or sieving are applied for aquatic systems [7], density separation for sediments [8,9], and chemical digestion for food or biota samples [3,10], in the latter two, MP particles are then collected on filters. The size cut-off of these

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Abbreviations	
AF4	Asymmetric Flow Field Flow Fractionation
AFM	Atomic Force Microscopy
ATR-FT-IR	Attenuated Total Reflection FT-IR
AUC	Analytical Ultracentrifugation
CE	Capillary Electrophoresis
CLSM	Confocal Laser Scanning Microscope
$d_g$	gyration diameter
$d_h$	hydrodynamic diameter
DLS	Dynamic Light Scattering
EDS	Energy Dispersive Spectroscopy
ELS	Electrophoretic Light Scattering
EM	Electron Microscopy
ENP	Engineered Nanoparticle
ESEM	Environmental Scanning Electron Microscopy
FFF	Field Flow Fractionation
FPA-FT-IR	Focal Plane Array FT-IR
FT-IR	Fourier-Transform Infrared Spectroscopy
HDC	Hydrodynamic Chromatography
HPLC	High Performance Liquid Chromatography
LD	Laser Diffraction
LOD	Limit of Detection
MALS	Multi Angle Light Scattering
MP	Microplastic (1 $\mu\text{m}$ –5 mm)
Nanoplastic	1–100 nm
NOM	Natural Organic Matter
NSOM	Near-field Scanning Optical Microscopy
NTA	Nanoparticle Tracking Analysis
PE	Polyethylene
PS	Polystyrene
PSD	Particle Size Distribution
Py-GC-MS	Pyrolysis Gas Chromatography Mass Spectrometry
RM	Raman Microspectroscopy
SEC	Size Exclusion Chromatography
SEM	Scanning Electron Microscopy
SPM	Scanning Probe Microscopy
STM	Scanning Tunneling Microscopy
Sub $\mu$ -plastic	Submicrometer-plastic (0.1 $\mu\text{m}$ –1 $\mu\text{m}$ )
TED-GC-MS	Thermal Extraction Desorption Gas Chromatography Mass Spectrometry
TEM	Transmission Electron Microscopy
TERS	Tip-enhanced Raman Spectroscopy
UC	Ultracentrifugation
UF	Ultrafiltration
WWTP	Waste Water Treatment Plant
XPS	X-ray Photoelectron Spectroscopy

methods, however, only covers the micrometer range (except for membrane filtration, Section 2.2.1). This is illustrated in Fig. 1. Hence, for sub $\mu$ - and nanoplastic there is a need for sampling that retains such small particles.

Following the sampling and sample treatment, there is the determination of the MP particle size and number – alternatively, the mass content – and the identification of the polymer. To that end, there are (i) techniques that combine microscopic and spectroscopic analysis and (ii) thermoanalytical methods [3,10,11]. In spectroscopy, MP can be measured on a particle by particle basis with attenuated total reflection Fourier transform-infrared spectroscopy (ATR-FT-IR) [10], where MP particles (usually larger than 500  $\mu\text{m}$ ) are handpicked. Smaller particles are collected on filters and identified with focal plane array micro-FT-IR (FPA-FT-IR) [10,12] or Raman microspectroscopy (RM) [13–15] with particle sizes down to 10  $\mu\text{m}$  and 1  $\mu\text{m}$ , respectively. Thermoanalytical MP detection, on the other hand, provides polymer and additive characterization and quantifies by mass content of MP in a sample. Its limits of detection (LODs) depend on the polymer type and are in the range of nanograms to micrograms [16–18].

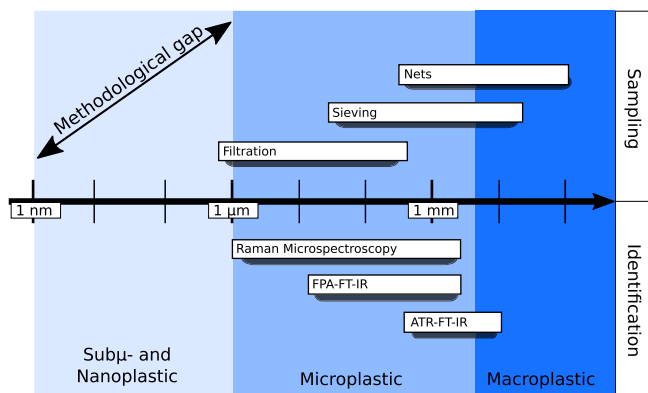


Fig. 1. The analysis of MP is established for particles down to 1  $\mu\text{m}$ . Below, there is a methodological gap.

It has to be kept in mind that the mass of the particle decreases with the third power of its diameter  $d^3$ . The orders of magnitude of the particle diameter  $d$  in m and particle mass  $m$  in g (assuming a density of 1) follow the correlation  $\log m = 3 \log d + 6$ . It gives the masses of e.g. 10  $\mu\text{m}$ , 100 nm and 10 nm particles as  $10^{-9} \text{ g} = 1 \text{ ng}$ ,  $10^{-15} \text{ g} = 1 \text{ fg}$  and  $10^{-18} \text{ g} = 1 \text{ ag}$ , respectively. This demonstrates the steep decrease of the particle mass and can be connected to the particle number, which, in turn, increases with  $d^3$ . This means that, with fixed mass, one 100  $\mu\text{m}$  plastic particle ( $m = 1 \mu\text{g}$ ) is equivalent to a thousand 10  $\mu\text{m}$ , a million 1  $\mu\text{m}$ , a billion 100 nm and a trillion 10 nm particles. From this consideration it can be concluded that, although unintuitive, sub $\mu$ - and nanoplastic can constitute high particle numbers and, at the same time, low masses in a sample (e.g. Refs. [1,2,19]).

Hence, techniques would have to provide low particle size detection limits or low LODs in terms of mass to detect sub $\mu$ - and nanoplastic in environmental samples. The size ranges for MP identification are plotted in Fig. 1, showing that for sub $\mu$ - and nanoplastic they have hit a limit. So have the thermoanalytical methods with LODs that are too high for the low masses of sub $\mu$ - and nanoplastic that we could expect in the environment.

This illustrates that for the analysis of sub $\mu$ - and nanoplastic we are facing a methodological gap. When entering the nanometer size range, a new approach in the analytical methodology must be taken. This concerns specific characteristics, like particle size distribution (PSD) or morphology and the chemical identity, for which techniques that detect in the nanometer range will be needed. In addition, an appropriate sample treatment, especially, a pre-concentration and also a separation step to properly isolate the particles, will be an essential part of the required protocol.

The established methods for MP analysis, however, have the potential to be adapted for the analysis of sub $\mu$ - and nanoplastic particles by combining them to other techniques. Such combinations, like pre-concentration to solve LOD issues or light scattering-based techniques that give a PSD to solve the problem of representativeness in techniques for single particle analysis, could alleviate their limitations. Furthermore, there is much knowledge on the analysis of ENPs in the environment that may potentially be

harnessed to close this methodological gap. A seminal paper by Hüffer et al. discussed similarities and differences that may help the understanding of micro- and nanoplastic particles [20]. Especially when regarding methods that concern particle characterization, there are many techniques for the analysis of ENPs that have been established [20–24]. Some of them may be transferred to nanoplastic analysis, mainly techniques that characterize particles by their geometry, like dynamic light scattering (DLS) or electron microscopy (EM). However, since ENPs are mostly inorganic and nanoplastic is mainly carbon-based, the identification of the chemical composition of the particle will require new protocols. Sub $\mu$ - and nanoplastic, as is usual in MP analysis, needs an identification of the particle composition in terms of the polymer type (e.g. polyethylene (PE), PS) instead of just an elemental analysis to provide a reliable characterization of the environmental sample.

Furthermore, first studies demonstrated the fragmentation of larger particles into sub $\mu$ - and nanoplastic. The tests were performed either on MP particles that were collected from the ocean [25] or pristine consumer products [26,27]. Two distinct steps in the formation of small plastic particles have been determined. First, a surface erosion of small, nano-sized particles. Second, fragmentation of the parent particles into equally sized smaller MP particles. The focus of other studies has been the propensity of sub $\mu$ - and nanoplastic to form heteroaggregates with organic and inorganic matter [28]. Primary nanoplastic beads and the dissolved organic content in seawater was studied, to show that nanoplastic accelerates the coagulation of dissolved organic matter to particulate organic matter [29].

These findings emphasize the great difference between ENPs and primary sub $\mu$ - and nanoplastic beads compared to secondary sub $\mu$ - and nanoplastic, which is likely to occur in the environment. Due to their fragmented nature, they will exhibit an irregular shape, surface morphologies and charges that are different from synthetic sub $\mu$ - and nanoplastic beads, on which many studies concerning the topic have been based. Here it is also important to mention their heteroaggregation behavior, which contrasts MP. The challenge for method development will be to accommodate for the properties that will be inherent to environmental sub $\mu$ - and nanoplastic particles.

Here we want to review the techniques that have already been applied in sub $\mu$ - and nanoplastic studies (search was conducted using Web of Science and Google Scholar with the search terms 'nanoplastic', 'nano- and microplastic' and 'analysis', amongst others, including publications until august 2018) and present techniques from ENP analysis that we think may have a potential for sub $\mu$ - and nanoplastic research. We will critically discuss their applicability and project a roadmap for the whole analytical process in environmental systems.

## 2. Sample preparation

Since plastic contamination can be found in very diverse locations, the analytical process begins with the clarification of the question that is to be answered (plastic particle number, size and PSD or mass of plastic particles per mass or volume of sample), which, in turn, depends on the sample to be analyzed. Samples can range from drinking water to food, and from environmental waters, sediments, biota tissue, to waste water treatment plant (WWTP) in-/effluents, which have greatly different contents of matrix that accompanies the plastic particles.

Plastic is an omnipresent material in our lives. Therefore, there is a strong risk of sample contamination during sampling and sample handling, which shows that proper particle contamination prevention measures have to be taken. Tools and setups should be made from non-polymer materials to avoid a systematic

contamination of the sample. Also, contamination due to airborne particles and synthetic fibers from clothing needs to be prevented by the use of laminar flow benches. However, it seems unlikely to completely avoid plastic in all components, therefore, a thorough blank value and recovery evaluation of the method should be performed.

### 2.1. Digestion of matrix

When choosing methods for sample treatment, characterization, and identification, in order to obtain the required information on the sample, in many cases it will be necessary to remove the matrix, in order to enable the technique to analyze the particles. This means organic matrix like tissue, organisms, or natural organic matter (NOM) has to be removed. Depending on the treatment, some inorganic particles may be removed as well, e.g. carbonate-based sediments with acid treatment.

A multitude of approaches for the digestion of organic matrix have been applied in ENP [21] and MP [3] analysis, some of which have also been utilized for nanoplastic [30]. These approaches comprise acid treatment, usually 65% nitric acid [30,31], sometimes in combination with 30% hydrogen peroxide [32,33] and alkaline treatment with sodium hydroxide [30,34,35]. Additionally, a mild enzymatic protocol with Proteinase K can be used for tissue decomposition [30,32].

The treatment must not alter the plastic particles, therefore care has to be taken when choosing the sample treatment protocol. Homogenization may damage the plastic particles. Also, it has been shown with optical microscopy and DLS that acid, alkaline and H<sub>2</sub>O<sub>2</sub> treatment cause aggregation of the particles, most likely due to the strong change in ionic strength of the solution [30]. Enzymatic treatment is milder and has been demonstrated to cause no [32] or less [30] aggregation with the particles (fluorescent, 100 nm, PS). Further, harsh treatments (acid, alkaline, H<sub>2</sub>O<sub>2</sub>) can, in some cases, negatively influence the fluorescence signal of labelled plastic particles (e.g. in toxicology studies) [30,33]. In many studies a proper validation of the digestion is lacking. It is, however, very important to find a sufficiently mild protocol that does not alter the plastic particles and stabilizes them against aggregation. If, on the other hand, information on the particle morphology and aggregation state is not required by the analytical question, this step can be reduced to a mere recovery test for quantification. For quantification of fluorescent particles, a recovery test concerning the stability of the fluorophore should be performed to evaluate a specific treatment, as well [33].

### 2.2. Preconcentration

Even though large amounts of plastic pollution are present in the environment [36], the mass of sub $\mu$ - and nanoplastic particles is probably very low [2,19]. Therefore, for most types of sample, a preconcentration step is inevitable. There are several different methods, from which may be chosen, depending on the sample and the following characterization and identification (Table 1).

#### 2.2.1. Membrane filtration

The process of filtration is very common for MP analysis, where e.g. the whole micrometer range of particles can be sampled on a filter membrane for spectroscopic analysis [3,37,38]. Membrane filters from different materials (e.g. aluminum oxide, ceramics, or polycarbonate) are commercially available with pore sizes in the range of several  $\mu$ m to 0.01  $\mu$ m. It has to be noted that the use of polymeric membranes could introduce plastic contamination to the sample. It should also be kept in mind that the size fraction in the filtrate is usually smaller than the nominal pore size. Membrane

**Table 1**

Methods for the preconcentration and separation (detectors are listed in Table 2) of sub $\mu$ - and nanoplastic particles. References are divided whether they have been applied for sub $\mu$ - and nanoplastic (left) and by documents from other fields (right).

Task	Technique	Range	Advantages	Disadvantages	References
Preconcentration	Membrane filtration	>10 nm	<ul style="list-style-type: none"> <li>Easily available</li> <li>Cheap</li> </ul>	<ul style="list-style-type: none"> <li>Low flow rates with small pores</li> <li>Small volumes</li> </ul>	[1]/–
	UF	10–100 kDa ca. 5–50 nm	<ul style="list-style-type: none"> <li>Large volumes</li> <li>Little sample damage/aggregation</li> <li>Little membrane clogging/fouling</li> </ul>	<ul style="list-style-type: none"> <li>Interaction with membrane</li> <li>Setup not plastic free</li> </ul>	[2,25,41,43]/–
	Dialysis	Similar to UF	<ul style="list-style-type: none"> <li>Mild conditions</li> </ul>	<ul style="list-style-type: none"> <li>Slow</li> <li>Large volume of counter dialyzing medium</li> <li>Risk of microbial contamination</li> <li>Harsh conditions</li> </ul>	–/[42]
	UC	Any	<ul style="list-style-type: none"> <li>Simple</li> <li>Washing of particles with centrifugation and redispersing</li> </ul>	<ul style="list-style-type: none"> <li>No separation from particulate matrix</li> <li>Difficult to obtain complete separation</li> </ul>	–/[21,42]
	AUC	1 nm–1 $\mu$ m	<ul style="list-style-type: none"> <li>High resolution</li> <li>Can provide many information</li> <li>Multiple detectors</li> <li>Cheap, easy</li> </ul>	<ul style="list-style-type: none"> <li>Best for small particles (1–10 nm)</li> </ul>	–/[49,50,67]
	Evaporation of solvent	Any		<ul style="list-style-type: none"> <li>Does not remove dissolved matter</li> <li>Superheating</li> </ul>	[51]/[42]
Separation	AF4	1 nm–1 $\mu$ m	<ul style="list-style-type: none"> <li>No stationary phase</li> <li>Sample focusing</li> <li>Online coupling</li> </ul>	<ul style="list-style-type: none"> <li>Operation difficult</li> <li>Interaction with membrane</li> <li>Steric inversion</li> </ul>	[32,41,55]/[53]
	HDC	5 nm–1.2 $\mu$ m	<ul style="list-style-type: none"> <li>Less interaction with stationary phase</li> <li>Coupled detectors</li> </ul>	<ul style="list-style-type: none"> <li>Little used</li> </ul>	–/[21]
	SEC	1 nm–100 nm	<ul style="list-style-type: none"> <li>Coupled detectors</li> </ul>	<ul style="list-style-type: none"> <li>Stationary phase</li> <li>Small range</li> </ul>	–/[62]
	HPLC	1 nm–40 nm	<ul style="list-style-type: none"> <li>Coupled detectors</li> </ul>	<ul style="list-style-type: none"> <li>Stationary phase</li> <li>Small size range</li> </ul>	–/[61]
	CE	5 nm–500 nm	<ul style="list-style-type: none"> <li>High separation resolution</li> <li>Coupled detectors</li> <li>Fast</li> </ul>	<ul style="list-style-type: none"> <li>Charge required</li> <li>Electrolyte/surface modification</li> <li>Interaction with capillary/clogging</li> <li>Might damage sample</li> <li>Complex matrices difficult</li> </ul>	–/[23,65,66]

filters need to be handled very delicately to avoid damaging of the membrane, which would compromise the size cut-off. When using filters with small pore sizes, especially below 0.1  $\mu$ m, they exhibit very low flow rates, which effects the sample volume that can realistically be filtered. This volume decreases with decreasing pore size (e.g. 250 mL with 0.4  $\mu$ m pores [39] or 0.1 mL with 0.01  $\mu$ m pores [1]). This needs to be considered when deciding on a treatment protocol for a large sample volume, especially when planning for environmental samples, which may contain high amounts of organic content in relation to the plastic particles, for which ultrafiltration (UF) could be a preferable alternative. On the other hand, since sub $\mu$ - and nanoplastic in the environment is expected to occur in hetero-aggregates with organic matter (Section 1) membrane filtration could retain and enable an imaging of those aggregates.

Membrane filtration in a five-step sequence of the pore sizes 25  $\mu$ m, 2.5  $\mu$ m, 0.45  $\mu$ m and two times 0.1  $\mu$ m has been performed for the isolation of PE nanoplastic particles (24 nm–52 nm) from facial scrubs [1]. This filtration cascade helped to avoid a fast clogging of the pores.

### 2.2.2. Ultrafiltration

UF uses nano-porous membranes that have a molecular weight cut-off in the range of 10–100 kDa (which roughly corresponds to 5–50 nm). They are employed either in a stirred cell [2], a centrifugal field [40] or in cross-flow mode (also called tangential flow) [41], in which the suspension flow is cycled past a parallel membrane, to prevent its blockage. As opposed to the methodologically similar dialysis [42], the filtration is performed by applying pressure to facilitate the flow of the filtrate, which increases its operation speed.

A stirred cell with a molecular weight cut-off of 10 kDa has been used to concentrate (factor 50) the sub $\mu$ - and nanoplastic fraction (<1.2  $\mu$ m) of artificially fragmented MP particles [25] and a marine water sample (factor 100) [2]. Cross-flow UF with a cut-off between 40 and 60 kDa has been evaluated as preconcentration (factor 200) step for asymmetric flow field flow fractionation (AF4) with PS beads in the range of 50 nm–1000 nm [41]. This indicates that Cross-flow UF is a method with high potential for the processing of environmental samples, because it is able to process large volumes of water, up to the m<sup>3</sup> range. It is also very gentle because it does not eliminate all the solvent, therefore particle loss and sample alteration or aggregation are minimized [21,43]. It has to be added that AF4 is also able to preconcentrate to some extent by creating a focusing flow that collects up to 50 mL of the sample at the beginning of the flow channel [44,45].

### 2.2.3. Ultracentrifugation

Centrifugation and ultracentrifugation (UC) can be used to sediment particles from suspensions into a pellet. For sub $\mu$ - and especially nanoplastic, the higher centrifugal forces of UC, which are in the range of 10<sup>5</sup> g, will be required to affect the smaller plastic particles, which bring the inherent difficulty of densities close to that of water. This technique is readily available and simple to use, but has the caveat that it only processes smaller sample volumes, in the range of 10–100 mL, which limits its applicability for environmental water samples. UC collects all particles in the pellet without separation, irrespective of whether they are plastic, or originate from the (in)organic environmental matrix. Additionally, the high centrifugal forces or stress from redispersing the pellet may alter the sample by forming aggregates or damaging plastic particles [21,42,46]. However, these drawbacks might be irrelevant

in an analytical protocol that is independent of the morphology of the sample, like pyrolysis gas chromatography mass spectrometry (Py-GC-MS) that destroys the sample anyway.

Employing density gradient UC [47], on the other hand, may provide a separation of plastic particles and matrix with higher densities, e.g. sediment [48]. This, however, will require a preceding digestion of organic matrix, which would otherwise be contained in the same fraction as the plastic particles.

There is also another variant called analytical ultracentrifugation (AUC) that spectroscopically (light absorption) monitors the sedimentation during the centrifugation process, from which many parameters on the morphology and spectroscopic information of the analyte can be derived. A fragmentation can be obtained as well [49,50].

#### 2.2.4. Evaporation of solvent

Evaporation of the solvent at reduced pressure, commonly with a rotary evaporator, is a technique from nanoparticle synthesis and especially useful for organic solvents [42]. It has, however, been applied for the concentration (factor 30) of environmentally more relevant, secondary PET nanoplastic suspensions (ca. 100 nm) in water, which were generated by laser ablation [51]. Since this technique does not remove dissolved matter and will be uneconomical for removing large volumes of water, it might be primarily applicable to further concentrate suspensions from e.g. dialysis or cross-flow UF that could only be reduced to a certain volume.

### 2.3. Separation techniques

#### 2.3.1. Field flow fractionation

Field flow fractionation (FFF) is a separation technique that applies a perpendicular force on particles in flow. Depending on their diffusivity, which is determined by characteristics like density or shape, particles are retained in the flow channel for different durations, which causes a separation of the particles in the sample. FFF works without a stationary phase, precluding interactions with the plastic particle, however, interactions with the membrane of the flow channel are a common problem that has to be countered by a proper method optimization for each individual sample. There are different variants of FFF, using various separating fields, like thermal, electric, gravity (or centrifugal), or cross-flow, which cover the whole nanometer range (for AF4) and can extend to the low micrometer range (up to 100  $\mu\text{m}$ ) [52,53].

The most common variant of FFF is the AF4, which, like other flow techniques, is routinely coupled to online detectors [53,54]. Frequently applied are refractive index [51], UV–visible absorption [41] or fluorescence [32] detectors for particle presence in the fraction and multi angle light scattering (MALS) [32,41,51] as well as DLS [54] that offer size characterization. Additionally, a mass spectrometric technique has been reported, that combines Py-GC-MS analysis with AF4 allowing for the determination of the polymer type [41].

AF4 (Table 1) has been widely utilized for the analysis of ENPs in environmental samples [53]. It has also been shown to be of great potential for the separation of sub $\mu$ - and nanoplastic samples: Gigault et al. published an AF4 method that is optimized for the fast separation of primary sub $\mu$ - and nanoplastic PS beads in the range of 1 nm–1000 nm [55]. Correia et al. evaluated a protocol for the analysis of primary nanoplastic particles (PS 100 nm) in a fish tissue sample, that digested the matrix with Proteinase K (which was shown to prevent aggregation, as opposed to acid treatment, Section 2.1) and separated the sample with AF4-MALS-Fluorescence [32]. Mintening et al. proposed AF4 and Py-GC-MS (Section 4.2) of samples that were preconcentrated by cross-flow UF for the analysis of plastic particles below 20  $\mu\text{m}$  as part of a framework for MP,

sub $\mu$ - and nanoplastic and validated the setup with primary PS particles in the range from 50 nm–1000 nm, suspended in drinking and surface water [41].

These methods have all been validated with primary sub $\mu$ - and nanoplastic particles. In contrast, plastic particles in the environment are generated due to various stressors, which fragment the particle and usually oxidize the polymer [56], therefore, the surface can be expected to be rough and negatively charged [27]. In addition, methods that have been developed for ENPs, will need optimization for sub $\mu$ - and nanoplastic particles due to their densities. These circumstances have to be accounted for in the method development of environmental sub $\mu$ - and nanoplastic by optimizing the dispersion medium, flow rates and validating the detectors. The validation also needs to include a determination of the concentration ranges, in order to perform a quantification [57].

When using AF4 for the separation of particles larger than around 1  $\mu\text{m}$ , the so-called steric inversion occurs. Here particles are drawn to the membrane almost equally strong but due to their size, large particles experience stronger forces from the laminar flow causing them to elute faster than smaller particles. The point of inversion depends on different parameters like channel thickness, flow rate or cross-flow [53]. This brings the risk of co-elution of small and large particles due to the steric inversion, which would compromise the separation. Therefore, a separation, e.g. filtration, step at the point of inversion is suggested [41].

As with the other separation techniques below, FFF is coupled on-line to detectors, which should also provide quantitative information on the plastic particles. This is possible with mass-spectrometric detectors or UV, refractive index, or fluorescence detectors [58] by calibration with a standard [59]. However, problems with particle-membrane interaction and limited concentration ranges [60], as well as providing a representative calibration material for the quantification of secondary sub $\mu$ - and nanoplastic will have to be addressed.

#### 2.3.2. Chromatography

Chromatographic techniques (Table 1) use a stationary phase for the separation of analytes, which make their applicability for particulate samples difficult, since interactions with the stationary phase may occur and their pore size may not suffice. On the other hand, their wide-spread and easier application make them a noteworthy alternative to FFF, which requires lots of method optimization and user experience. Some techniques have been applied for ENP separation in environmental samples; among them are reverse phase high performance liquid chromatography (HPLC) [61], size exclusion chromatography (SEC) [62] and hydrodynamic chromatography (HDC) [63]. This indicates that they may be applicable to nanoplastic separation as well. However, in contrast to ENPs, sub $\mu$ - and nanoplastic particles, which originate from fragmentation, can be expected to have rougher surfaces, which may increase their interaction with the stationary phase. Another point to consider is the difference in density between ENPs and sub $\mu$ - and nanoplastic, for which a stable suspension has to be ensured.

Many of the chromatographic methods, however, may only have an application for a specific analytical question, since the particle size range is much smaller (1 nm–40 nm for HPLC [61] and 1 nm–100 nm for SEC [62]) as compared to e.g. FFF (Fig. 2) [21]. Concerning these restrictions, HDC might be a chromatographic method that is applicable for sub $\mu$ - and nanoplastic particles because its stationary phase is a non-porous material, packed into the column, in which the mobile phase flows through the inter-spaces. This brings a larger size range (5 nm–1.2  $\mu\text{m}$ ) [63] and

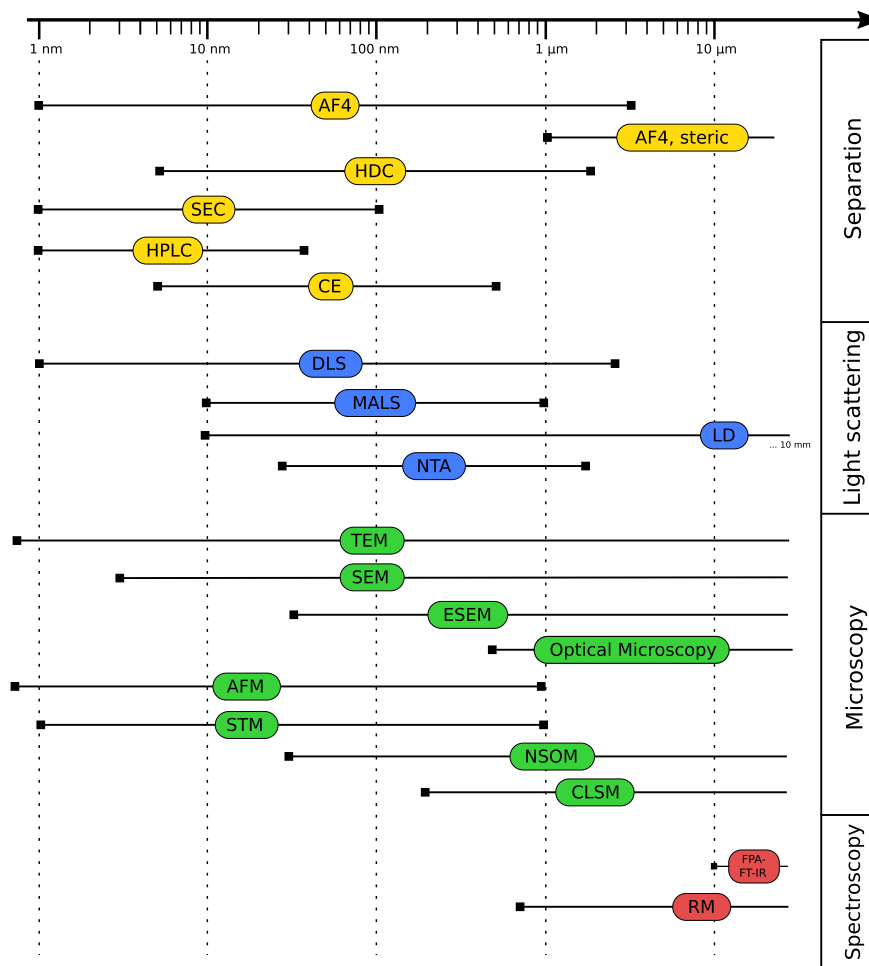


Fig. 2. Comparison of the particle size ranges for analysis with the different techniques that are discussed in this review.

less interactions. In a study on Au ENPs (5 nm–100 nm), HDC has shown better recoveries than AF4, although its separation resolution was smaller [64].

### 2.3.3. Electrophoresis

Electrophoresis (Table 1) employs the mobility of charged particles in an electric field to achieve a spatial separation. This review will focus only on capillary electrophoresis (CE) because it has the largest range of applicability as compared to e.g. gel electrophoresis [65,66]. CE has been applied on ENPs, indicating that it has the potential to be a promising alternative for the separation of sub- $\mu$ - and nanoplastic particles. This method will also have to be adapted to the nature of environmental sub- $\mu$ - and nanoplastic, especially its surface properties, which will depend on the influences that the particles have experienced.

In all the separation techniques of this chapter, the suspension has to be stabilized with surface functionalization agents. For CE they are also necessary to precisely control the surface charge [21]. These surfactants may, however, impede subsequent characterization of the nanoplastic particles.

## 3. Methods for the characterization of particle size and morphology

### 3.1. Particle characterization by light scattering

There are multiple methods that apply the scattering of laser light on particles to obtain information on physical properties like

size or PSD (Table 2). The most widely used, DLS, measures particle sizes in the range from 1 nm to 3  $\mu$ m based on the fluctuation of intensity of a laser beam that passes the suspension. This fluctuation is caused by the Brownian motion of the particles and can be associated to the hydrodynamic diameter ( $d_h$ ) of the particles with an autocorrelation function [68]. Its easy application makes DLS a broadly used technique for particle size and PSD characterization, especially for primary nanoplastic, which is used for spiking and toxicological experiments.

It should, however, be kept in mind that DLS uses theoretical models that are based on spheres and that it works optimally with monodisperse suspensions. This is due to the fact that the signal intensity correlates with  $d^6$  and, therefore, overestimates large particles. The technique is, thus, prone to errors due to contaminations that introduce large particles, which would mask the actual analyte. Such contaminations may be residues from the matrix, aggregates or dust [21,22]. However, gaining reliable information on polydisperse, non-spherical sub- $\mu$ - and nanoplastic dispersions, as would be present in the environment, is nonetheless possible [2,55]. When employing any of these light scattering detectors, especially for quantification, a preemptive method validation should contain a determination of the concentration range [69]. In addition, DLS does not provide any chemical information and cannot distinguish particles of similar form but different composition.

Commercial DLS instruments are usually coupled with the function to determine the zeta potential with electrophoretic light scattering (ELS). The principle of ELS is similar to DLS, in that it

**Table 2**  
Techniques for the characterization and imaging of sub $\mu$ - and nanoplastic particles. References are divided whether they have been applied for sub $\mu$ - and nanoplastic (left) and by documents from other fields (right). Concentration ranges quoted from Ref. [68].

Task	Technique	Information	Range/Limits	Advantages	Disadvantages	References
Characterization	DLS	Size ( $d_h$ ), PSD, aggregation behavior	1 nm–3 $\mu$ m conc. $10^{-6}$ – $10^{-1}$	<ul style="list-style-type: none"> <li>Fast, cheap</li> <li>In situ</li> <li>Non-invasive</li> <li>Aggregation</li> <li>Direct coupling</li> </ul>	<ul style="list-style-type: none"> <li>Large particles</li> <li>Polydispersity</li> <li>Complex matrix</li> <li>Non-spherical particles</li> </ul>	[28,29]/[68]
	ELS	Surface charge, stability	1 nm–3 $\mu$ m	<ul style="list-style-type: none"> <li>Fast, cheap</li> <li>Non-invasive</li> <li>With DLS</li> </ul>	<ul style="list-style-type: none"> <li>Electro-osmotic effect</li> <li>Sensitive to environment</li> </ul>	[28]/[68]
	MALS LD	Size ( $d_g$ ), PSD Size	10 nm–1000 nm 10 nm–10 $\mu$ m conc. $10^{-5}$ – $10^{-1}$	<ul style="list-style-type: none"> <li>Online coupling</li> <li>Large size range</li> <li>Easy, fast</li> <li>Automated</li> </ul>	<ul style="list-style-type: none"> <li>Requires clean samples</li> <li>Only spherical model</li> </ul>	[41,55]/[53,54,68] -[68]
	NTA	Size ( $d_h$ ), PSD, number concentration	30 nm–2 $\mu$ m conc. $10^{-6}$ – $10^{-5}$	<ul style="list-style-type: none"> <li>Better with polydisperse samples</li> <li>Complex media</li> <li>Particle corona</li> <li>High resolution</li> <li>Precise size information</li> </ul>	<ul style="list-style-type: none"> <li>Complex in operation</li> <li>Spherical model</li> </ul>	[26,27]/[69,70]
Imaging	TEM	Size, shape, aggregation, imaging	<1 nm	<ul style="list-style-type: none"> <li>High resolution</li> <li>Precise size information</li> </ul>	<ul style="list-style-type: none"> <li>Quantification difficult</li> <li>Sample preparation</li> <li>Expensive</li> </ul>	[5]/[21,22,24,75]
	SEM	Size, shape, aggregation, imaging, surface morphology	ca. 3 nm	<ul style="list-style-type: none"> <li>High resolution</li> </ul>	<ul style="list-style-type: none"> <li>Quant. difficult</li> <li>Sample preparation</li> <li>Charging effects</li> </ul>	[28]/[21,22,24,74,75]
	ESEM	Size, shape, imaging, surface morphology	ca. 30 nm	<ul style="list-style-type: none"> <li>Wet samples,</li> <li>Environmental conditions,</li> <li>Non-conductive samples</li> <li>Complementary to EM</li> </ul>	<ul style="list-style-type: none"> <li>Reduced resolution</li> </ul>	-[21,22,24,74,75]
	EDS	Elemental composition	nm range		<ul style="list-style-type: none"> <li>Elemental information not sufficient</li> <li>Diffraction-limited</li> </ul>	-[21,22,24,74,75]
	Optical Microscopy & Fluorescence Microscopy	Size, shape, morphology Particle Location	> 1 $\mu$ m	<ul style="list-style-type: none"> <li>Non-destructive</li> <li>Cheap, easy to handle</li> <li>Sub-diffraction variants</li> </ul>	<ul style="list-style-type: none"> <li>Environmental plastic is not fluorescent</li> </ul>	[72,73]/-
	AFM	Size, shape, topography, aggregation	ca. 0.1 nm	<ul style="list-style-type: none"> <li>High resolution</li> <li>AFM-IR</li> <li>TERS</li> <li>In liquid</li> <li>High resolution</li> </ul>	<ul style="list-style-type: none"> <li>Slow</li> <li>Small area</li> <li>Artefacts due to particle movement</li> </ul>	-[22,24]
	STM	Size, shape, topography, aggregation	ca. 1 nm		<ul style="list-style-type: none"> <li>Conductive samples</li> <li>Slow</li> <li>Small area</li> </ul>	-[22,24,79]
	CLSM	Size, shape, location in	> 0.2 $\mu$ m	<ul style="list-style-type: none"> <li>Fluorescence imaging</li> </ul>	<ul style="list-style-type: none"> <li>Small area</li> <li>Diffraction limit</li> </ul>	[72,88–90]/-
NSOM	Size, shape	ca. 30 nm	<ul style="list-style-type: none"> <li>Fluorescence</li> </ul>	<ul style="list-style-type: none"> <li>Slow</li> <li>Small area</li> </ul>	-[22,24,91]	

measures the fluctuation of laser intensity that is generated by particle movement in an electric field. This gives the electrophoretic velocity that, in turn, correlates to the zeta potential, which is the charge of the particle shear surface. The zeta potential is a measure on the stability of a suspension, with values greater than  $\pm 30$  mV being considered stable against aggregation [22,68]. Since plastic particles in the environment will experience weathering [56], they will display oxidized surfaces [27] and with that changes in surface charge. Measuring the zeta potential can, therefore, be an important parameter to characterize the ageing of plastic particles.

Static light scattering (SLS), which is also called MALS, records scattered laser light at different angles to obtain information on the size of the particle, e.g. the radius of gyration  $d_g$ . To provide precise size information, a monodisperse suspension is needed [21,68]. Therefore, MALS is commonly coupled online to AF4 [32,41,51,55] to feed size-separated samples to the detector (Section 2.3.1), which enables measurement of polydisperse, environmentally relevant, sub $\mu$ - and nanoplastic particles [55].

Laser diffraction (LD) is another static laser scattering-based technique that is common in process analysis, which is capable of sizing (among others) solid particles in liquid media across a very large size range from 10 nm to 10  $\mu$ m [68]. It would, therefore, be an interesting technique for characterizing both MP, sub $\mu$ - and nanoplastic at the same time.

Nanoparticle tracking analysis (NTA) records scattered laser light with a microscope and a digital camera. Software processing tracks the motion, as recorded in the video, of the particles and correlates a hydrodynamic diameter due to its Brownian motion [21,26,70]. NTA suffers less perturbation from large particles in polydisperse samples [69], which makes it an alternative to DLS for the determination of PSD of sub $\mu$ - and nanoplastic. This has already been shown for fragmented, i.e. secondary plastic [25–27]. Another consideration for the application of NTA for sub $\mu$ - and nanoplastic could be to use its fluorescence mode in combination with particle staining, which would, however, have to be tested for interference with the preceding sample digestion.

### 3.2. Imaging

To obtain information on the morphology of a sample, microscopy is the most viable method because it offers direct access to its geometry and surface characteristics. There are many different operation modes of microscopes, from which three groups are most prominent in particle imaging: optical microscopy, EM and scanning probe microscopy (SPM). These types of microscopy utilize different sorts of interaction with the sample, which effect their resolution (Table 2).

### 3.2.1. Optical microscopy

Optical microscopy is a ubiquitous tool in almost every laboratory and is an essential part of the single particle analysis of MP particles, i.e. micro-IR and RM. For the visualization of sub $\mu$ - and nanoplastic, however, optical microscopes can only be applied for particles above the diffraction limit of 0.3  $\mu\text{m}$ –0.5  $\mu\text{m}$  depending on the numerical aperture and the criterion for single point differentiation [71]. Some techniques like fluorescence or RM (Section 4.1.1) utilize a confocal pinhole to block light from lateral parts of the focal point, thereby enabling a lateral and axial resolution close to the diffraction limit.

Fluorescence microscopy in combination with fluorescently marked particles is a common tool to analyze the behavior of (usually primary) sub $\mu$ - and nanoplastic in organisms, e.g. to track the translocation of the particles in the tissue. Such an experiment has e.g. been performed with fluorescent PE MP in the Antarctic krill that breaks fluorescent reference particles down to secondary sub $\mu$ -plastic particles with its mastication apparatus. The particles can be further translocated into different parts of the organism [72,73].

Even though fluorescence microscopes are diffraction limited, as well, it is possible to locate smaller particles as long as they emit sufficient fluorescence signal. This application is, however, limited to synthetic particles with fluorescent dyes. It could not be used for the analysis of environmental sub $\mu$ - and nanoplastic because these particles do not usually contain fluorophores.

### 3.2.2. Electron microscopy

In EM [74,75] the sample is scanned with an electron beam that is generated in an electron gun and focused with electron optics. The electrons display a wide variety of interactions with the sample that can be observed with different detectors and give specific and complementary information. Since the wavelength of the high-energy electrons is very short compared to the wavelength of visible light of optical microscopy, the resolution of EM is much higher, spanning the range from sub-nanometers to millimeters. Its high resolution makes EM a widely used technique that complements many studies with imaging information on nanometer-sized particles.

Scanning electron microscopy (SEM) uses lower acceleration voltages up to 30 kV, usually around 5 kV, which causes varied interactions with the surface of the sample and accordingly detection being (in an angle) above the sample. In most cases, imaging is performed by detecting lower energy secondary electrons whose emission from sample atoms has been caused by the electron beam and gives morphological information of the point of incidence. The electron beam can also eject an inner shell electron of a sample atom, whose replacement is accompanied with the emission of an element-specific X-ray photon that is registered on an energy dispersive spectroscopy (EDS) detector and gives information on the elemental composition of the irradiated part of the sample.

Transmission electron microscopy (TEM) detects the transmitting electron beam below the sample, which requires high electron acceleration voltages up to 300 kV and a very thin sample. This results in very high resolutions enabling the imaging of very small nanoparticles. Due to the transmission mode of operation, TEM provides information on the interior of the particles rather than the surface (for which SEM can be utilized). It, too, can be coupled with EDS or electron energy loss spectroscopy (EELS) for information on the elemental composition [21].

TEM and SEM are often applied to characterize size, shape and surface characteristics of sub $\mu$ - and nanoplastic particles, very commonly in studies that use primary nanoplastic that is pre-characterized before being used to spike samples for method validation [32] or biota exposition experiments [76]. They have also

been utilized to image secondary sub $\mu$ - and nanoplastic, e.g. from fragmentation studies [25,26]. The strength of SEM to image surface topology has been used to analyze the heteroaggregation behavior of nanoplastic with natural organic and inorganic matter [28,77]. EM is sometimes applied to deduce a PSD [51,78] of a sub $\mu$ - or nanoplastic sample, which, however, is prone to error, since the imaged section may not be representative for the whole sample and the number of measured particles may be too low.

In general, EM requires a sample preparation that makes the sample stable for high vacuum and precludes artefacts from charging due to the electron irradiation. Here, drying a sample dispersion on a suitable substrate, e.g. for TEM carbon coated Cu grids, is the common approach. This preparation, however, alters the sample and may induce aggregation of the particles or shrinkage of organic matter or bacteria. Furthermore, coating with a metal (Au, Pt, Ag) or carbon film is often applied in order to avoid a charging of the sample.

Environmental scanning electron microscopy (ESEM) is a variant of SEM that allows the analysis of environmental, wet samples in a low pressure (10–50 Torr) nitrogen atmosphere, thereby enabling the imaging of samples that would otherwise degrade in high vacuum. The detector of the ESEM mode is not based on direct secondary electron detection, but on the detection of an ion cascade caused by the interaction of the electron with the low-pressure atmosphere, therefore, non-conductive samples may be imaged without charge artefacts, even without prior metal coating.

Another method to image samples in their environmental condition, e.g. aggregation or the particle corona, is Cryo-EM. Here, a dispersion of the sample is very rapidly frozen with liquid nitrogen or liquid ethane, causing water in the sample to vitrify instead of crystallizing. Hence, water does not expand and preserve the sample integrity. This technique has been used to determine the agglomeration behavior of PS nanoplastic particles in the presence of NOM and different salts [77]. During the Cryo-SEM analysis and image acquisition, it is important to remember that the sample preparation may produce spherical droplets of vitreous ice with a diameter of few micrometers or below, which look similar to plastic particles. To preclude incorrect attributions, a confirmation of their identity is very important e.g. by EDS or by heating and sublimating ice from the sample.

### 3.2.3. Scanning probe microscopy

A third group of microscopy that is not governed by the diffraction limitation of incident light is SPM [22]. With these, a sensor, in some cases a sharp tip, scans the surface of the sample and detects the interaction with it. For scanning tunneling microscopy (STM) this is the tunneling current between the conductive surface and the tip in close proximity, which is dependent on the distance, hence giving a topographic image [79]. Its application to nanoplastic may, therefore, be problematic, since the particles itself are not conductive. Atomic force microscopy (AFM) records the deflection of a cantilever carrying the tip, which is caused by electrostatic or van der Waals interactions, that generate a height profile of the sample [22,24]. AFM is able to analyze samples in liquid, e.g. the adsorption behavior of nanoplastic particles with the cell wall of green algae [80], however, characterization of the particle surface roughness may be influenced by the tip geometry and needs to be accounted for by data processing models [81]. It can also be combined with IR [82–84] or Raman spectroscopy [85–87] to create chemical images of the samples (Section 4.1.1 and 4.1.2).

Another group of SPMs that are based on optical microscopy contain, on the one hand, the confocal laser microscope (CLSM, alternatively LSCM), which is a confocal optical microscope that scans the sample with a resolution at the diffraction limit (down to



about 200 nm), usually exciting and detecting fluorescence. CLSM is often used to investigate the translocation of fluorescently dyed nanoplastic particles in organisms [72,88–90]. And on the other hand, there is near-field scanning optical microscopy (NSOM, alternatively SNOM) that conducts laser light through a tip aperture. This aperture is smaller than the laser wavelength, which causes the light to evanesce in a strongly localized area in close proximity of the tip, hence producing sub-diffraction limit resolution [91]. Due to their resolution these techniques have the potential for sub $\mu$ - and nanoplastic imaging.

These techniques offer high resolution but have the drawback of long and laborious measurements, which can only be performed for specific particles or sections of the sample, but not for a representative analysis. This makes SPMs, as well as the other types of microscopy, dependent on proper sample treatment and characterization to guarantee representativeness.

#### 4. Chemical identification

The chemical identification of the polymeric particle is of great importance for the analysis of sub $\mu$ - and nanoplastic in environmental samples, because, on the one hand, it provides a confirmation of the analytical question i.e. the sub $\mu$ - and nanoplastic presence in a system. On the other hand, it can provide an additional chemical characterization of the particle e.g. with information on additive presence and/or ageing (Table 3). In MP analysis, vibrational spectroscopy has usually been integrated with optical microscopy to provide imaging, which enables the analysis of individual particles. This gives a great amount of information on particle size, PSD, and geometry, as well as the spectroscopic identification. This methodology, however, becomes increasingly difficult with decreasing particle size (below 10  $\mu$ m) due to (i) an increase in the amount of small particles and (ii) particle size detection limits of the method [3,14].

In sub $\mu$ - and nanoplastic analysis, this calls for a combined approach of techniques that provide information on size and PSD (Section 3) and techniques that can give spectroscopic identification. This, in turn, eliminates the need for single particle analysis with the spectroscopic methods and only demands a bulk measurement (of e.g. a dried fraction), in case when the preceding sample treatment has generated a sufficiently pure (and mono-disperse) sample fraction.

Micro-FT-IR and RM are being more and more automated and provide information on the amount of particles, their size, and PSD through their microscopy images, as well as chemical identity [15,92]. Their optical resolution is, however, limited by the wavelength of the light source, which restricts them to the micrometer range.

#### 4.1. Spectroscopy

##### 4.1.1. Infrared spectroscopy

FT-IR [12,93] is the most common spectroscopic technique in MP analysis [94], in which the irradiation of a sample with infrared light excites vibrational transitions, whose absorbance gives a specific so-called fingerprint spectrum that provides identification of the sample. With polymers, it is even able to display ageing by observation of surface oxidation via characteristic bands (e.g. carbonyl). FT-IR is applied mainly in two different modes of operation: attenuated total reflection FT-IR (ATR-FT-IR) which is used for handpicked MP (>500  $\mu$ m) [10] and focal plane array (FPA) detector-based micro-FT-IR (FPA-FT-IR) which images MP particles on filters with a resolution of ca. 10–20  $\mu$ m [10,12].

FT-IR can only be applied for the bulk analysis of sub $\mu$ - and nanoplastic, since the size limit for FT-IR single particle analysis is in the range of 10  $\mu$ m [3,10,12,95]. Such an analysis has already been done to confirm the identity of nanoplastic contained in facial scrubs as PE, by drying the filtered suspension and performing an ATR-FT-IR measurement on the powder [1]. This, however, requires a few mg of dried particles, in this study the suspension that was dried for the measurement amounted to around  $10^{10}$  particles. Another method for measuring the sub $\mu$ - and nanoplastic particles is by producing a KBr pellet containing the sample [76,96]. FT-IR, as well as, RM (Section 4.1) is capable of identifying mixtures, which produce an overlay of the respective spectra of each individual substance. Nonetheless, deconvoluting a spectrum of a multitude of (possibly impure) polymers, which may be the case with an environmental bulk sample, poses a significant challenge in data processing. Here, a separation technique may provide alleviation, if the setup can achieve (partial) separation of the polymers, by e.g. density or material-dependent surface properties.

There exists a commercial set-up that combines FT-IR with AFM, in order to be able to record chemical images with a spatial

**Table 3**  
Techniques for the chemical identification and characterization of sub $\mu$ - and nanoplastic particles. References are divided whether they have been applied for sub $\mu$ - and nanoplastic (left) and by documents from other fields (right).

Technique	Information	Range	Advantages	Disadvantages	References
FPA-FT-IR	Vibrational spectrum, Pigments, Additives, Ageing	>10 $\mu$ m	<ul style="list-style-type: none"> <li>• Non-destructive</li> <li>• Automated</li> </ul>	<ul style="list-style-type: none"> <li>– Not applicable for single sub<math>\mu</math>- and nanoplastic</li> <li>– Strong interference from water</li> </ul>	[1,41]/[10,12]
ATR-FT-IR	Spectrum, imaging	Bulk	<ul style="list-style-type: none"> <li>• Simple, fast</li> <li>• High resolution</li> <li>• Chemical imaging</li> <li>• Non-destructive</li> <li>• Easy sample preparation</li> <li>• Fast</li> <li>• No interference from water</li> <li>• Surface characterization</li> </ul>	<ul style="list-style-type: none"> <li>– Slow</li> <li>– Small area</li> <li>– Fluorescence</li> </ul>	–/[82,83]
AFM-IR		>50 nm			
RM	Fingerprint spectrum, Pigments, Additives	>0.5 $\mu$ m, Bulk			–/[3,14]
XPS	Binding energies of orbitals	Bulk		<ul style="list-style-type: none"> <li>– UHV</li> <li>– Laborious</li> </ul>	[1,51]/[99]
Py-CG-MS	Mass Polymer type Additives	Bulk LOD: ng – $\mu$ g e.g. PS: LOD: 4 mg/L	<ul style="list-style-type: none"> <li>• Little sample preparation</li> </ul>	<ul style="list-style-type: none"> <li>– LOD dependent on polymer type</li> <li>– Some polymers difficult</li> <li>– Dry sample needed</li> <li>– Preconcentration necessary</li> </ul>	[2,41]/[16,17]
TED-GC-MS			<ul style="list-style-type: none"> <li>• Measurement with matrix</li> <li>• Fast</li> <li>• Higher sample masses</li> </ul>		–/[18]

resolution below the diffraction limit. Here IR absorption at the location of the tip causes thermal expansion, which induces an oscillation of the AFM cantilever tip. With that, spectral and spatial information in the range of 50 nm can be obtained [82–84]. AFM-IR can be an interesting method for the analysis of nanoplastic particles, mainly for the analysis of special samples and specific particles. Since the method images selected areas, a representative analysis of many particles could be hard to realize.

#### 4.1.2. Raman microspectroscopy

Besides FT-IR, there is RM [13–15], which employs the inelastic scattering of laser light that gives a vibrational fingerprint spectrum. This spectrum is complementary to the FT-IR spectrum and also enables the unambiguous identification of the plastic particles. Since the light source does not need to be infrared light, shorter wavelength lasers (e.g. 532 nm) can be utilized, which results in higher spatial resolution. RM is, therefore, applied for the analysis of MP particles down to 1  $\mu\text{m}$  [10,14,97,98].

RM has the potential for single particle analysis of sub $\mu$ -plastic, because, as mentioned in Section 3.2.1, Raman microscopes are confocal, giving them submicrometer resolution. Although this resolution enables the single particle analysis of sub $\mu$ -plastics, it would take several days to identify a representative amount of particles. Therefore, bulk measurements after preceding particle separation and characterization would provide a reliable sub $\mu$ - and nanoplastic analysis, which could easily be complemented with a bulk FT-IR analysis.

As with AFM-IR, Raman spectroscopy has also been coupled to AFM for nanoscale imaging with spectroscopic information at spatial resolutions of 10 nm [85–87]. In tip-enhanced Raman spectroscopy (TERS), the tips are coated with Ag or Au and are able to generate Raman signal enhancements due to localized surface plasmons and the lightning rod effect [87]. TERS has been used to investigate polymer-blend interactions in thin films [85], which suggests that it may be applied for environmental sub $\mu$ - and nanoplastic analysis. This, however, remains to be seen, since the surface plasmon signal enhancement is strongly dependent on the distance from the sample and the probe tip. Hence organic matter on the surface of the environmental plastic particle may obstruct a Raman spectroscopic identification.

#### 4.1.3. X-ray photoelectron spectroscopy

In X-ray photoelectron spectroscopy (XPS) the sample is irradiated with X-radiation that causes the emission of photoelectrons, which contain information on their binding energy, hence giving element-specific characteristic bands. XPS valence band analysis allows the assignment of the binding energies of different orbitals, which are influenced by their chemical structure (e.g. methylene vs. methyl groups) [99]. XPS spectra have been used to confirm the chemical composition of a primary sub $\mu$  PS latex [96] and primary PE nanoplastic particles in cosmetics [1]. It has to be noted, though, that XPS alone may not be able to unambiguously identify the polymer type. However, it has the capability to observe changes in the surface oxidation of sub $\mu$ - and nanoplastic particles via changes in the oxygen content of the sample [51,96]. Further, XPS has been utilized to investigate the changes in the extracellular polymeric substances of WWTP microorganisms, which were induced by nanoplastic particles [76].

#### 4.2. Gas chromatographic-mass spectrometric methods

Besides spectroscopy, the mass spectrometric polymer identification is another common approach in MP analysis that gives information on the mass fraction of a polymer rather than particle count. There are two different methods. The first is Py-GC-MS, in

which the sample – usually a handpicked MP particle with a mass of up to 350  $\mu\text{g}$  [100] – is thermally degraded in an inert atmosphere with the result that the pyrolysis fragments of the polymer structure can be separated by gas chromatography and characterized by mass spectrometry. Hence, polymers are identified by typical pyrolysis products [16,17,101]. The second GC-MS-based approach, thermal extraction desorption gas chromatography mass spectrometry (TED-GC-MS) allows the analysis of plastic in environmental samples (sample mass of up to 20 mg [18]) without removal of (in)organic matrix. It combines thermal extraction of thermogravimetric analysis products onto a solid-phase adsorber, which comprises the main difference to Py-GC-MS. The pyrolysis fragments are subsequently thermally desorbed into gas chromatography mass spectrometry to enable the identification of the polymer [18,102]. Py-GC-MS has recently been used for the first observation of sub $\mu$ - and nanoplastic (<1.2  $\mu\text{m}$ ) in the environment, i.e. in the North Atlantic Subtropical Gyre. Comparison to a reference database and principle component analysis enabled the detection of PE, PS, polyethylene terephthalate, and polyvinylchloride in the sub $\mu$ -plastic fraction [2]. Py-GC-MS has also been validated for the identification of sub $\mu$ - and nanoplastic particles (PS, 50 nm–1000 nm) in a protocol that combines cross-flow UF, AF4 and Py-GC-MS [41].

These techniques, however, suffer from LODs, which will be too high for sub $\mu$ - and nanoplastic detection without preconcentration. Mintening et al. reported a LOD of 4 mg/L for a PS nanoplastic suspension, which they could decrease by a factor of 200 to 20  $\mu\text{g}/\text{L}$  by concentration with a cross-flow UF [41]. A preconcentration will be essential for the detection of sub $\mu$ - and nanoplastic, because its mass in environmental samples can be expected to be very low (Section 1). Since no data on sub $\mu$ - and nanoplastic content in the environment is available, it is difficult to project the demands to LODs and preconcentration factors. However, in the one instance, where sub $\mu$ - and nanoplastic has been detected in the North Atlantic Subtropical Gyre with Py-GC-MS, a preconcentration by a factor 100 sufficed to enable a detection of characteristic decomposition products [2].

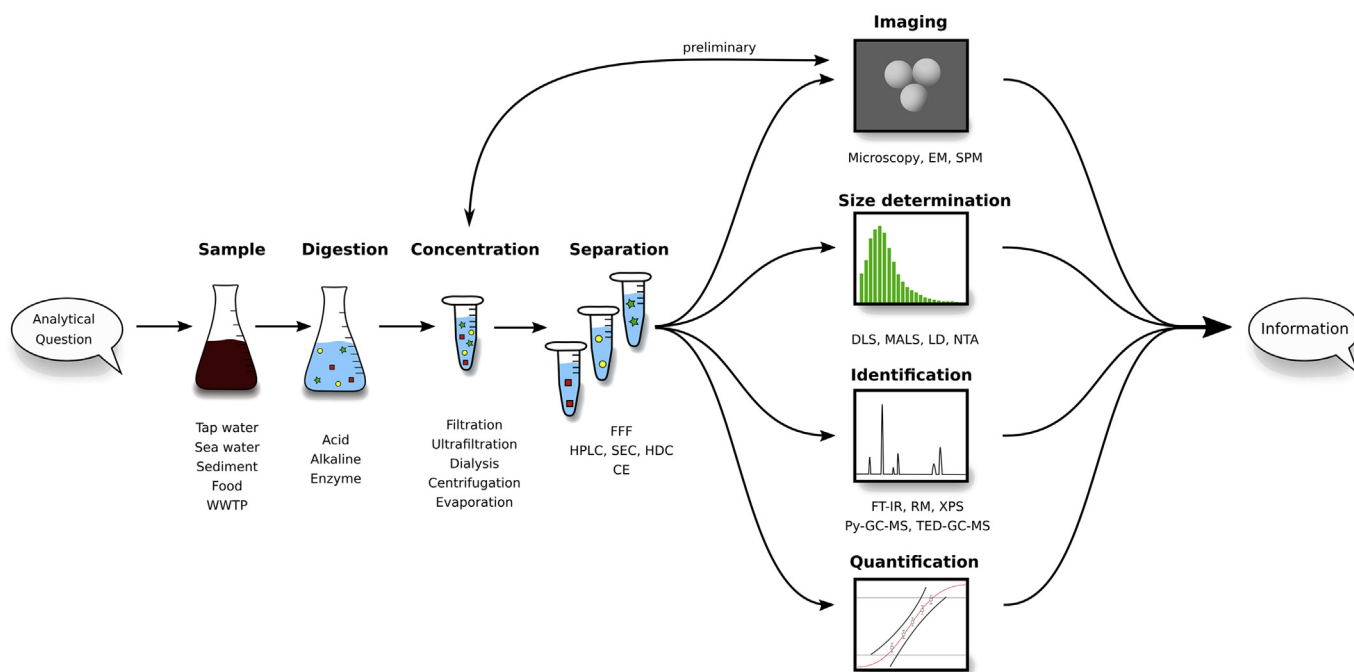
Py-GC-MS and TED-GC-MS are faster than spectroscopic single particle analysis but cannot provide information on particle size, number or PSD, as well as morphology or aggregation, which will have to be obtained by other particle characterization techniques like AF4-MALS or DLS. This emphasizes the need to combine different methods to generate the specific information, which is demanded to answer the individual analytical question (Section 5).

### 5. Roadmap

When regarding MP analysis, there is no single protocol but a multitude that covers a complex field, requiring specific methods for each different type of sample. This is also true for sub $\mu$ - and nanoplastic analysis. Therefore, the essential first step is to clearly define the analytical question. This contains the sampling scenario, like surface water, tap water, food, soil, or WWTP in-/effluent (Fig. 3), which, in turn, determines all subsequent steps, in particular, the sample treatment to ensure that the sample can be analyzed by the different techniques. It should be noted that, initially, when a method is transferred to an environmental sample, a preceding validation will be necessary.

The analytical question also defines the required information: Are detailed size parameters and geometry of importance or is a pure mass content sufficient information? Is chemical information on the polymer required? When a clear task has been set, the appropriate methods can be chosen.

If the sample contains too much organic matrix that would disrupt further analysis, a digestion of the matter is necessary.



**Fig. 3.** Roadmap for the analytical process of sub $\mu$ - and nanoplastic particles. Starting from the sample in its matrix, we present and discuss the single steps that will lead to a full sub $\mu$ - and nanoplastic analysis.

Acidic, alkaline, or enzymatic methods have been presented (Section 2.1). In case of samples without organic matrix, like drinking water, sample processing can be continued with the preconcentration step. Presumably, preconcentration is necessary for virtually all samples because – due to the small particle size – sub $\mu$ - and nanoplastic content is always low when evaluated by mass. For this step, we discussed membrane filtration, UF, UC and mere removing of the solvent (Section 2.2). Here the desired information dictates the method of choice. If the PSD is to be studied, e.g. by DLS, sensitive UF should be utilized; if only SEM and spectroscopic identification are planned, filtration on a membrane filter could suffice; if a mass content is to be determined, a pellet from UC might be appropriate without further separation.

Following the concentration, a separation can isolate sub $\mu$ - and nanoplastic from organic or inorganic particles in other size fractions, for which we discussed field flow fractionation, chromatographic and electrophoretic methods (Section 2.3). If a sediment-free and digested sample is analyzed, so that only sub $\mu$ - and nanoplastic particles should be left, the separation step could be omitted. In the present literature on sub $\mu$ - and nanoplastic, AF4 has been the separation technique of choice because it has no stationary phase, and because of its large size range and its online coupled detectors [32,41,51,55].

After the analyte has been isolated, a morphological characterization and chemical identification can be performed. For this, many different physicochemical parameters can be determined. Among these are size and PSD, shape, surface morphology, surface charge, degree of aggregation, surface functionalization, and chemical composition [22,23]. Which of them will be determined and with that, which methods will be applied depends on the research question. For data connected to particle size and PSD we discussed light scattering methods (Section 3.1) and for the imaging of the particles and their morphology, optical, electron and scanning probe microscopy (Section 3.2).

When applying methods for particle size and PSD characterization, the inherent differences between the spherical,

monodisperse reference particles and environmental, secondary sub $\mu$ - and nanoplastic have to be accounted for with a proper method validation. Environmental plastic is mostly generated by fragmentation, which causes the particles to have irregular shape and surface morphology, as well as a different surface charge as particle standards. However, the break-down of macroplastic to sub $\mu$ - and nanoplastic will involve a substantially larger number of fragmentations, which should make the structural variety that we see in MP (e.g. fibers, sheets) less pronounced. Furthermore, these particles in the nanometer range will show increased Brownian motion and, therefore, be recognized by most methods by their hydrodynamic diameter rather than the actual shape. Therefore, the methods of Section 3 can, in many cases, be applied for the physical characterization of irregular sub $\mu$ - and nanoplastic samples (e.g. Refs. [25–27]).

If the sample is polydisperse or suffers contaminations, laser scattering methods like DLS will lose accuracy. This emphasizes the need for a combined analysis with different techniques. Separation techniques like AF4, especially when coupled online, would alleviate the polydispersity and contamination problem by providing a monodisperse particle fraction at the moment of passage through the detector.

To characterize the morphology of the irregularly shaped particles, an imaging technique should complement the characterization. It should also be noted that, if possible, a preliminary imaging before deciding on a preconcentration and separation step could be well advised, because the morphology of secondary sub $\mu$ - and nanoplastic can impact the efficiency of the individual methods (see Fig. 3).

These methods for particle characterization and imaging, however, cannot provide information on polymer type. Finally, to obtain the chemical identification of the particle with respect to polymeric composition, single particle analysis or bulk measurements of a fraction can be performed, for which we presented spectroscopic and mass spectrometric methods (Section 4). Therein, depending on the analytical question, it is possible to obtain different

information. Aside chemical identification of the polymer type, additives (e.g. stabilizer and pigments), as well as ageing can be determined. Most usually, though, a simple and fast distinction between polymer and natural particle is the core requirement of the methods. Here, the thermoanalytical methods (Section 4.2) are currently the faster ones, considering that the microspectroscopic techniques perform single particle identification (on MP), which increases measurement time. When entering the realm of sub $\mu$ - and nanoplastic, however, particle characterization can be performed by other, better suited, techniques (Section 3). This can make it sufficient to perform a spectroscopic bulk analysis on a plastic particle fraction, thereby reducing the measuring from thousands of spectra to a few, possibly making the time requirement equal to thermoanalytical methods. This directs the criteria for the choice of the method for chemical identification away from measurement time and towards the information, which the specific method can provide and its ability to be coupled to other techniques.

One such instance has been published by Mintening et al. in which MP, sub $\mu$ - and nanoplastic particles in aquatic samples can be detected and quantified. This framework connects the analysis of MP (by sieving/filtration and FT-IR) with the analysis of sub $\mu$ - and nanoplastic, which is performed by preconcentration with cross-flow UF and analyzed with AF4-MALS and Py-GC-MS [41]. The size threshold for the change from FT-IR detection to AF4 and Py-GC-MS detection is 20  $\mu$ m. Between this and ca. 1  $\mu$ m, the AF4 is operated in inversion mode. To separate the sample for the analysis in normal mode and inversion mode, a filtration step at 1  $\mu$ m is performed. This approach has the advantage of a fast FT-IR imaging, but requires two AF4 separations and Py-GC-MS identifications. Another protocol could be based on RM (Fig. 3), which can perform MP analysis down to 1  $\mu$ m, and, thus, would be more time consuming but could eliminate the AF4 separation in steric mode and also provide information on the single particle level for the entire MP range. This indicates that for the diverse analytical questions of sub $\mu$ - and nanoplastic analysis, a versatile toolset will be of the essence.

## 6. Conclusion

Sub $\mu$ - and nanoplastic pose new challenges to the methodology of environmental plastic analysis. In the chapters above, we discussed methods for the analysis of sub $\mu$ - and nanoplastic, which have already been applied. In addition, from the field of environmental ENP analysis and from MP analysis, we discussed techniques, which have the potential to be transferred to plastic particles in the nanometer range. We emphasized the need to adapt the analytical protocol to the sample and the required information by selecting the appropriate techniques (Fig. 2). This is important since sub $\mu$ - and nanoplastic particles can be characterized by many different parameters, which influence their behavior, so that one technique alone is unlikely to give a sufficient characterization.

Environmental analysis requires cost efficient and fast methods, which can handle a large number of samples in order to facilitate the evaluation of contamination and risk assessment. This emphasizes the need for optimized protocols that are tailored to quickly providing the required information on each sample, unless an academic interest warrants a full (and, therefore, time-consuming) characterization of a sample. It is, however, necessary to select and combine techniques that provide the minimal amount of data to answer the analytical question. This road to routine analysis will benefit from on-line coupling (e.g. AF4-UV-MALS, combined with a chemical identification) to enable reliability and high throughput. For this, we presented various techniques that

have the potential for sub $\mu$ - and nanoplastic analysis and projected a roadmap for the whole analytical process.

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